

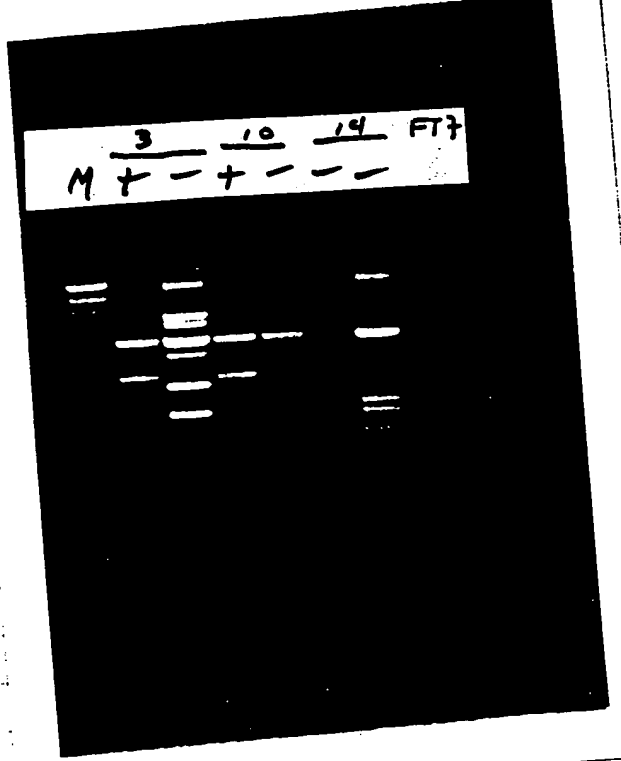
~~Exhibit~~ 3

2/2 Digest FT7 DNA 3, 10, 14 w/ KpnI/HindIII  
use previous prep as control (-)

Expected Size  
(3) Sense (4) AntiS. (-)  
3159 3159  
1573 1366  
625 782

10 3159 3159  
1573 1121  
650 832  
380 650

14 3159 3159  
1573 945  
650 832  
204 650



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Major problem w/ #14

Some how samples got mixed-up

Go back & check out - Start for single colonies

A D: E

Also mini prep from original culture  
Digest w/ KpnI/HindIII 0/14

4/23 Run gel of Digests

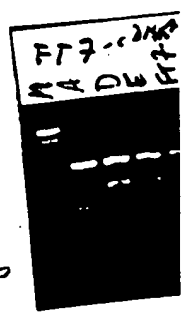
Clearly Sample A which was given as mate prep is  
in the wrong orientation

Start 0/14 of D: E to mini prep before  
start of 500ml culture

That mini prep FT7 DNA 14-D: E

Digest w/ KpnI/HindIII

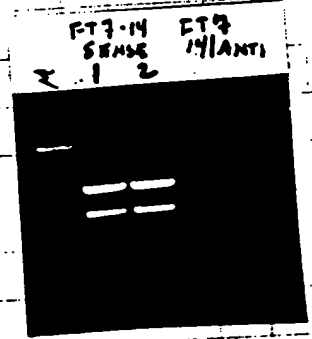
run on gel - Both are fine  
use -D for mate



M D E

7/25 Plasmid isolation of FT7, cDNA 14 - D up to Binding  
 - do 500 nls in 2 250 sets, end w/ 2 tubes to Band  
 7/26 Pull Bands - double band 1 pop - 6 hrs during the day

7/27 Digest FT7, cDNA 14 w/ Kpn I / Hae III  
 ① - single bandy  
 ② Double Bandy  
 ③ FT7, cDNA 14 antisense



Check Absorbance / Concentration of

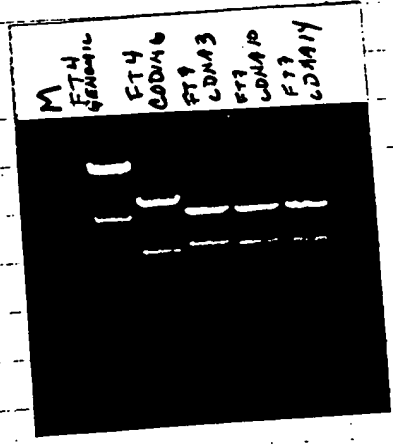
Samples	260	FT7 cDNA 14	280
①	.127	.062	.63 ng/μl 2.0
②	.143	.065	.71 ng/μl 2.3

Sequence  
 FT7, cDNA 3  
 T7  
 cDNA 14  
 T7  
 8850  
 9007  
 9874

Digest Mouse FT4

	260	280	260/280
Pst I genomic	.085	.045	
Not cut	.106	.060	

Wash about Cooling  
 Digest cDNA 3  
 Kpn I cDNA 10  
 Hae III cDNA 14



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# 8/4 FACS Analysis of Transfected cells w/ following vectors

pCDNA 7

FT7, 1, 2a, 2b, 3

1, 2b, 3

1, 3

cDNA 3

cDNA 10

cDNA 14

2 plates / Vector - Divide

FACS 7mls

Yuko 5mls

EAT 3mls

(7.5ul)

ETassy 5mls

(100ul)

Antibodies

IgM H - black 1:100

IgM hrf green 1:1000

IgM SLrf 1:200

IgG ha red 1:500

IgG SLa blue 1:500

2nd Antibody

IgM 2.5mls

12.5 / 2.5mls

IgG 1.5

60 / 1.5mls

Results are

H - all neg

hrf - all neg

SLrf, pCD (-), 1, 2a, 2b, 3(+), 1, 2b, 3(+), 1, 3 (-), cDNA 3 (-), 10 (+), 14 (+)

ha all neg

SLa all neg

8/8 Sygma 12 pum KG FT4

6451

6080

2470

6199

6374

6087

6306

6086

6203

6085

5721

5671

8/9 Run sy gel of above samples

Also sygma

5728

6084

7213

5731

5737

6082

5662

5727

6201

6200

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8/10 Sequencing gel of 8/9 samples  
Sequence

FT4 6079  
6202  
6307  
6373  
CDNA10 715  
946

CDNA14  
T7  
8850  
8807  
8874

Protein assay of FACS Samples, Also CAT assay

pcDNA

FT7-1,2a2b,3

FT7-1,2b,3

FT7-1,3

CDNA3

CDNA10

CDNA14

BSA Blank Protein

0 1.00 116 108

1 0.201 225 214

2 0.369 383 376

4 0.691 673 682

8 1.215 1.230 1.222

16

Sample

pcDNA .292 .294 .293

1,2a2b,3 .337 .330 .33

1,2b,3 .298 .343 .32

1,3 .369 .372 .37

CDNA3 .363 .379 .3

CDNA10 .306 .298 .3

CDNA14 .225 .253 .2

FACS Results

Only stain w/ shx

1,2a2b,3 23.6%

1,2b,3 24.6%

CDNA10 14.9%

CDNA14 8.0%

# Micro BCA Protein Assay

Reagent mic	MC	MB	MA
Per assay tube (ml)	0.01	0.24	0.25
Cocktail for Tubes			

Incubate 1 h at 60°C and cool to room temp.  
Since the color development has no end point, all tubes must be heated and cooled at the same time

1 mg/ml BSA (l)	Water (l)	Reagent (l)	Abs. 562	
0.0	500.0	500.0	Blank	Slope = 0.0734 Y intercept = 0.0656 X intercept = -0.8940 R = 0.9985
1.0	499.0	500.0	0.108	
2.0	498.0	500.0	0.214	
4.0	496.0	500.0	0.376	
8.0	492.0	500.0	0.682	
16.0	484.0	500.0	1.222	

8/10 Sequencing of FT4 9/10 Samples

Sample	l in assay	Water (l)	Reagent (l)	Abs. 562	mg protein/ml
pcDNA1	5.00	495.00	500	0.293	0.62
FT7 1,2a2b,3	5.00	495.00	500	0.333	0.73
FT7 1,2b,3	5.00	495.00	500	0.320	0.69
FT7 1,3	5.00	495.00	500	0.370	0.83
cDNA 3	5.00	495.00	500	0.371	0.83
cDNA 10	5.00	495.00	500	0.302	0.64
cDNA 14	5.00	495.00	500	0.239	0.47

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9/11 CAT Assay (FT7) samples  
2.5 µl of cell extract  
Control  
pcDNA  
1,2a2b,3  
1,2b,3  
1,3  
cDNA 3  
cDNA 10  
cDNA 14

Cocktail 15  
3H Chlase 300  
Tris, 2M pH 8.0 75  
But Co A 75  
H<sub>2</sub>O 300  
50/ tube

CAT

Vector		Counts/5ul	8/12/94		Incorporated Counts (.95)		Total Counts		Total Counts Incorporated	
pcDNA1			11,349	11,829	9,189	8,063	238,169	244,643	9,673	8,487
FT7 1,2a2b,3			11,161	11,441	27,211	21,919	250,431	250,739	28,643	23,073
FT7 1,2b,3			11,772	11,826	37,541	40,684	272,981	277,204	39,517	42,825
FT7 1,3			11,215	11,690	23,076	28,706	247,376	262,508	24,291	30,217
cDNA 3			11,834	11,206	33,885	39,096	270,565	283,216	35,668	41,154
cDNA 10			12,017	11,312	30,066	33,165	270,406	259,405	31,648	34,911
cDNA14			11,079	11,570	44,133	40,529	265,713	271,929	46,456	42,662
Control				10,354		424		207,504		446
	Protein Conc. (ug/2.5ul)	Total Counts Inc- Bkg			% INC/hr		% INC/hr/ug			Mean CAT Activity
pcDNA1	1.55	9,249		8,063	3.92	3.30	2.53	2.13		2.33
FT7 1,2a2b,3	1.83	28,219		22,649	11.27	9.03	6.17	4.95		5.56
FT7 1,2b,3	1.72	39,093		42,401	14.32	15.30	8.33	8.89		8.61
FT7 1,3	2.07	23,867		29,793	9.65	11.35	4.66	5.48		5.07
cDNA 3	2.07	35,244		40,730	13.03	15.47	6.29	7.48		6.88
cDNA 10	1.60	31,224		34,487	11.55	13.29	7.22	8.31		7.76
cDNA14	1.17	46,032		42,238	17.32	15.53	14.81	13.28		14.04

9/12 Assemble Data of FT7 FACS, CAT Assay / give to Judy  
Work on FT4 sequence

9/15 7 days sequencing on TruScribe FT4 samples  
6451 6200  
6378 6079  
6306 6307  
6203 1899  
5721 1898  
1899

9/16 Sequencing gel of 8/15 samples (FT7) Formamide gel  
Probe Thomas' rather than w/ GAP probe  
To check condition of RMT

9/18 The 7 days technique didn't resolve all of the compressions  
Try a Terminal transferase technique  
Run standard synerase rxn, after extension made  
Heat tubes (A, C, G, T) for 1.5 mins 100°C  
Hold on ice 10 min, Purpore TdT / dNTP cocktail  
Add to tubes, 37°C 30 min  
Add 5 µl Stop